Isostaticity and controlled force transmission in the cytoskeleton - A model awaiting experimental evidence

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Abstract

A new model is proposed for force transmission through the cytoskeleton. A general discussion is first presented on the physical principles that underlie the modelling of this phenomenon. Some fundamental problems of conventional models - continuous and discrete - are examined. It is argued that mediation of focused forces is essential for good control over intracellular mechanical signals. The difficulties of conventional continuous models to describe such mediation are traced to a fundamental assumption rather than to them being continuous. Relevant advantages and disadvantages of continuous and discrete modelling are discussed. It is concluded that favouring discrete models is based on two misconceptions which are clarified.

The model proposed here is based on the idea that focused propagation of mechanical stimuli in frameworks over large distances (compared to the mesh size) can only occur when considerable regions of the CSK are isostatic. The concept of isostaticity is explained and a recently developed isostaticity theory is briefly reviewed. The model enjoys several advantages: it leads to good control over force mediation; it explains nonuniform stresses and action at a distance; being continuos it makes it possible to model long-scale force propagation; it enables prediction of individual force paths. To be isostatic or nearly so, cytoskeleton networks must possess specific structural characteristics and these are quantified explicitly.

Finally, several implications of the new model are discussed. In particular, it is suggested that the mechanism for static force transmission may give insight into the dynamics of reorganisation of the CSK. Many of the results are amenable to experimental measurements, providing a testing ground for the proposed picture.

Introduction

Many cell functions, including growth, motility, gene expression, apoptosis and signal transduction, are controlled by mechanical stresses and alterations in cell shape [1]. Forces are transmitted between the interior of cells and extracellular matrix through transmembrane receptors, the most relevant of which for the peresnt discussion are probably integrins [2]. Within the cell stresses are mediated to a large extent by the cytoskeleton (CSK) - a network of actin filaments (AFs), intermediate filaments (IFs) and microtubules. This filamentous structure extends throughout the cytoplasm. The receptors link to the CSK at focal adhesion points effectively linking the nucleus to the surface of the cell.

Conventional models treat the cell as a membrane enclosing a continuous internal medium that may be elastic, viscous fluid, or visco-elastic [3], but this approach has been questioned in recent years. There is evidence that such descriptions of the cell do not capture key features of the mechanics of cells, such as the nonuniformity of strain distribution inside the cell [6] and channeled propagation of force stimuli over large distances across CSK networks[7]. These phenomena cannot be accounted for by conventional continuous descriptions without resorting to complex anisotropic constitutive properties correlated over large distances across the cell. This led to suggestions that the intracellular mechanics may be better understood if the effects of the discreteness of the CSK were taken explicitly into consideration [4][5]. Of particular interest to the thrust of this paper are observations of 'action at a distance', namely that upon pulling a bead attached to a receptor in the membrane of a cell, the relatively distant nucleus changes shape in response[7]. This type of response suggests that localised force stimulation of membrane receptors can be communicated reliably to the nucleus without grossly distorting the entire cell.

While the implication of the discreteness of the CSK structure in occurrence of nonuniform stresses appears to make sense on some conceptual level, it nevertheless introduces several difficulties. One is that stress propagation through discrete networks is sensitive to the structural details of the network and therefore quantitative predictions of discrete models would depend strongly on model premises. Another difficulty, which is hardly addressed in the literature, is that nonuniform force transmission, as observed in [7] for example, requires particular network structural characteristics, as will be discussed in detail below. A third problem has to do with the methodology of science in general. A model should provide accurate predictions, for example on the details of the intracellular response to force stimuli at the membrane. But to compute the forces at every structural element of the CSK is a hopeless task - the CSK network not only contains many filamentous elements, but its structure also changes constantly. The default description of many-element systems is by continuum coarse-grained models and blaming the continuous nature of conventional models in the failure to account for the observed phenomena, leaves one with a difficult decision of the introduction of an alternative. These problems have severely hindered the development of a first-principles predictive model of stress transduction in the CSK.

It is in this context that one should view the controversy that arose following the proposal of Ingber and coworkers that the CSK may be described as a tensegrity structure [8][9]. The term tensegrity, short for tensional integrity, had been coined by R. Buckminster Fuller in the 1920's and it refers to stable structures of force-carrying stiff rods and tight strings [10]. Although the idea of the CSK as a tensegrity structure has been forwarded already in the mid 1980's, its usefulness has remained controversial. One critique of it is that even on the static level it is difficult to translate this idea into detailed predictions on intracellular propagation of stresses. Another problem is quite fundamental - tensegrity cannot really explain nonuniform stresses on scales much larger than the mesh size of a framework, an issue that will be discussed in more detail below. Consequently, tensegrity toy models [5][11] are too small to be useful on a quantitative level and predictions made on the basis of descriptions of the CSK as ordered lattice networks [12] are not only unrealistic, but also miss a key point, as will be made clear below.

The claim of this paper is that the aforementioned experimental observations have implications that go well beyond the idea of tensegrity and may call for a shift of paradigm altogether. The central aim of this paper is the resolution of the above difficulties and the development of a generic model for the transmission of forces in the CSK. The structure of this paper is as follows. First, a general discussion is presented on the issue of cell control over mediation of forces from a designer's point of view. This discussion elucidates several advantages of transmission of channeled forces through the CSK. Next, the requirements of the model to be developed are specified. These provide clear criteria against which models in general, and this one in particular, have to be tested. The performance of conventional continuous models is then examined against the requirements. Two fundamental premises, which appear to underlie the recent drift in preference towards discrete models, are identified as misconceptions and put straight.

This preliminary discussion forms the basis for the main proposal of this paper - that a natural way to achieve the functionality requirements and to explain the observations of nonuniform stresses and action at a distance is by large regions of the CSK being isostatic or near-isostatic. Isostatic states are then defined and a brief review is presented of isostaticity theory. Explicit solutions for stresses in two-dimensional isostatic systems are presented, demonstrating that forces can be communicated controllably from the boundary into the cell. Next, a quantitative analysis is developed of the structural conditions that the CSK must satisfy in order to be isostatic. Due to lack of data several possible scenarios are analysed. This analysis makes it possible to test the isostaticity idea by direct observations of particular statistical properties of the CSK and potential such experimental tests are suggested. The results are summarised in the concluding section and some implications of the model for the reorganisation dynamics of the CSK are suggested.

Control over stress transmission

Cells constantly need to respond to mechanical stimuli applied at receptors on the membrane. In many cases the response involves adjustment of the internal structure, where the nature of the adjustment strongly affects the functionality of the cell. Consequently, shape changes must be controlled to a very good precision. Shape changes are directly linked to the way that intracellular stresses are mediated and the understanding of the control mechanism that govern mechanical stress transmission in the cell has been one of the central quests in the field.

Suppose one is required to design such a mechanism from fresh. What would be the principles that guide such a design? Efficiency would require several basic features: (i) minimal complexity; (ii) a response time that is short relative to other processes in the cell; (iii) the force communicated to the nucleus should relay as much information as possible about the position and the nature of a faraway stimulation (e.g. at the membrane); (iv) the information should be communicated with *as little attenuation as possible.*

A transmission mechanism based on an initial mechanical response satisfies both requirements (i) and (ii); it is simpler to manage than the coordination of an array of biochemical reactions and it takes place over timescales that are much shorter than chemical reactions which rely, amongst other factors, on slow diffusion. While it is clear that biochemical reactions play an important role in intracellular mechanotransduction, the difference in timescales relegates them to trail an initial mechanical response. Thus, stimuli at the membrane would give rise to mechanical response in the CSK, this response would stimulate biochemical activity, which may in turn generate further mechanical modifications. A complete description of the response over a wide range of timescales must include the combined effects of both mechanisms and the interplay between them. The construction of such a comprehensive theory is beyond the scope of this paper. Rather, we concentrate on the development of a fundamental model of the control through mechanical response at short timescales. The hope is that such a model will serve as a stepping stone to more complex versions that would include the mechano-chemical aspects. A brief discussion of possible implications of the model for the biochemical reorganisation of the CSK will be presented in the concluding discussion.

To satisfy requirement (iii), it should be possible to determine, at the locus of the nucleus, both where force stimuli originate at the membrane and the characteristics of the stimulating force. The simplest way to achieve this is by retaining *directional* information; extending a line from the nucleus in the direction that the force comes from gives information about the rough location on the membrane where the stimulus acts.

Requirement (iv) is essential because cells have many functionalities that take place simultaneously and give rise to 'noisy' environments. A signal transmitted from the membrane has to travel all the way to the neighbourhood of the nucleus and still be detected above the noise. This places restrictions both on the largest possible rate of attenuation of the signal and on its magnitude. One way to maintain a large magnitude is by keeping the transmitted forces focused as they propagate through the CSK away from the memnbrane (see figure 1).

Having specified the requirements of the system, let us now consider what would be regarded as a good model. First and foremost, the model should give the best possible combination of specifications (i)-(iv), which should allow a good level of precision in the communications of forces across the CSK. Second, the model should be able to explain experimental observations, in particular the difficult ones: nonuniform distribution of stresses in the cell and action at a distance. Third, it should go beyond qualitative explanations and conceptual understanding and provide quantitative predictions against which it could be tested. Ideally, such predictions should include the forecast of the spatial distribution of forces that develop in the CSK.

This means that, given a force stimulus at a particular location at the membrane (say, point A in figure 1), the model should be able to predict, at least in principle, whether the force is felt at point B and its magnitude at that point. As will be discussed in the concluding section, this requirement is also important in understanding the effects of the static picture on the dynamic reorganisation of the CSK.

The fourth requirement touches at the heart of the ongoing controversy: should the model be discrete or continuous? This issue is not straightforward and requires some discussion. Continuous models are the default description of most physical phenomena. This is in spite of the fact that, at some basic level, whether molecular, particulate or celestial, systems are made of discrete elements. The main reasons for this are: (i) practicality - one is often interested in behaviour on lengthscales (and correspondingly timescales) that are much larger than the discrete elements and therefore the wealth of data on the discrete level is often more prohibitive than it is helpful - and (ii) convenience - analysis of continuous functions is easier for theorists then the manipulation of discrete functions. Thus, whenever possible, the first attempt at modelling any physical system of many ingredients has always been on the continuous level. But this should not be done at all cost. For example, it would be useless to describe continuously phenomena that take place on the scale of the discrete mesh size. A continuous model is a coarse-grained description that is useful only when the measurable, or interesting, phenomena are on lengthscales much larger than this size. Thus, to construct a continuous model it is essential to first understand the dominant mechanisms on the discrete level and then build a continuous description that is a faithful representation of those mechanisms.

Vis-à-vis the requirements listed above, conventional continuum models do not seem to perform well. The main argument against them is that they give rise to *uniform* propagation of stresses from the point of force stimulus (e.g. point A in figure 1b). This both disagrees with observations and dissonates with requirements (iii) and (iv). However, to construct better models one has to first analyse the reason for this shortcoming. The following argument demonstrates that problem is quite fundamental and it lies in the premises that are built into the equations of conventional models. Specifically, it is the premise that continuous stress equations must involve information on deformations. Such information could be either in the form of stress-strain relations, in the case of static models, or in the form of stress-strain rate relations, in the case of viscoelastic models. This idea is paradigmatic in current modelling of mechanical stresses and it can be traced back to Saint-Venant [13], who introduced compatibility conditions in order to calculate stresses in elastic solids. Since elasticity theory is the flagship of this paradigm it is convenient to illustrate the problem within this context and in two dimensions.

In mechanical equilibrium the continuous stress field must satisfy force and torque balance conditions, which in two dimensions can be written in the form

$$\begin{aligned}
\sigma_{xy} &= \sigma_{yx} \quad \text{(torque balance)} && (1)\\
\frac{\partial \sigma_{xx}}{\partial x} + \frac{\partial \sigma_{xy}}{\partial y} &= g_x \quad \text{(force balance in x)} && (2)\\
\frac{\partial \sigma_{xy}}{\partial x} + \frac{\partial \sigma_{yy}}{\partial y} &= g_y \quad \text{(force balance in y)}\ . && (3)
\end{aligned}$$

In these equations σ_{ij} are the components of the stress tensor $(i, j = x, y)$ and $\vec{g}(\vec{r})$ is an external force field applied to the medium. Body forces can be disregarded in the following analysis without loss of generality. The three equations (1)-(3) are not enough to solve for the *four* components of the stress tensor σ_{ij} and Saint-Venant proposed to close the set of equations in two steps. First, impose a compatibility condition that the material remain continuous under defromation. In two dimensions this provides one additional equation that relates the second derivatives of the *strain field.* Second, introduce a further *independent* constitutive relation between the stress and the strain. The same procedure works in three dimensions. There are *nine* components of the stress tensor to determine, but only *six* balance conditions - three on forces and three on torque moments. The equations required to close the set are provided by imposing compatibility conditions, which inter-relate the second derivatives of the strain field, and then supplementing these conditions with stress-strain relations.

The involvement of the gradients of the strain field has a crucial consequence - it makes the stress field equations *elliptic.* It is this very feature that causes the uniformisation of the stress field when a localised force (a stimulus) is imposed at a given point, as sketched in figure 1b. This type of solutions not only disagrees with observations of action at a distance, but also clashes with requirements (iii) and (iv) in that they attenuate along any path from A to B and make it difficult to identify at B the origin of the force stimulus. Thus, it is the use of compatibility conditions that undermines the use of continuous elasticity for the description of intracellular stresses. The above argument can be readily extended to visco-elastic models since those use compatibility and strain-rate information to close the stress equations. Not surprisingly, such models are also hard-pressed to explain highly nonuniform stresses.

Thus, we arrive at a significant conclusion: the attenuation of stresses in existing models stems from a basic principle built into the equations, *not from the description of the stress as a continuous field.* To sharpen this conclusion, it should be noted that not all continuous models lead automatically to stress uniformisation. For example, hyperbolic equations, such as those discussed later in the paper, give rise to solutions that propagate nonuniformly along characteristic lines. It follows that continuous models may not be as inadequate as believed and their rejection on the grounds of being continuous could be misguided.

Let us consider now the other end of the argument - the seeming advantages of discrete models. Other than the discontent with continuous models, the main drive behind the move in this direction was the seemingly intuitive idea that discrete structures make it possible to explain both the observations of nonuniform intracellular strains and action at a distance. This idea is based on a presumption that, merely by being discrete, structures can retain directional information and propagation of forces in a focused manner. But this is a misconception. In most known discrete structures focusing of forces can only take place on the scale of the mesh size - over larger distances this is no longer the case. After all, all materials are made of discrete elements at some basic scale, yet most materials often display dispersion of stresses on macroscopic scales as if they are ideally elastic of visco-elastic.

The action at a distance, observed in the CSK over distances much larger than the mesh size, implies that this presumption needs to be carefully examined. In other words, the mere introduction of discreteness in the description does not guarantee resolution of the above conundrums on the scale of the entire CSK.

In view of these conceptual problems and the inherent benefits of continuous modelling discussed above, the loss of faith in a good continuous model for stress transmission in the CSK may be premature.

The above discussion gave well defined requirements that a model of intracellular stress transmission should satisfy and led to the conclusion that, although conventional continuous models fall short, continuous modelling should not be precluded in general. To be useful, a continuous model must give rise to a set of non-elliptic equations and the question is whether a physically plausible such a description can be constructed. Ideally, we should start from a discrete picture that supports action at a distance and coarse-grain it consistently to the continuum whilst retaining this feature.

The key to a successful such approach is the observation that discrete frameworks transmit forces over distances in a focused manner if they are in *isostatic states.* The definition of these states and the way that stress is transmitted in isostatic media are discussed next.

Isostatic states and isostaticity theory

A stable structure of discrete elements is in an isostatic state when all the forces that its components exert on one another can be determined from statics alone. Such structures are known in engineering as *statically determinate.* Equations of statics involve only balance conditions - of forces and of torque moments. A highschool textbook example of such a structure is a ladder of a given weight standing on a rough floor and leaning against a smooth wall (see figure 2). The floor exerts on the ladder forces in the x and y-directions while the wall exerts on the ladder only a force in the x-direction. In mechanical equilibrium the three forces can be determined by three balance conditions on the ladder - two of force and one of torque moment. A point to note is that the determination of the forces requires no information whatever about the constitutive properties of the material that makes the ladder, e.g. its Young's modulus.

The same is true for systems of many 'ladder-like' elements - static determinacy is independent of the number of elements, which can be as large as 10^{12} in some materials. The seemingly straightforward observation that the forces can be determined regardless of the compliance of the structural constituents has a striking consequence. Balance conditions contain information only about spatial distribution of forces, whilst compliance is information that relates the forces to deformations. This means that in isostatic structures the forces are independent of any deformation-based information. Now, the stress field is only a continuous representation of the field of discrete forces. It follows that information about strains or strain rates is *redundant* for the determination of stresses in such media.

But, as discussed above, this type of information is at the foundation of elasticity, elasto-plasticity and visco-elasticity. The conclusion is that the field equations of conventional theories are inadequate to describe isostatic states. This already gives a first indication that isostatic networks may not transmit stresses like conventional materials.

Whether a framework is in an isostatic state depends on its structural characteristics and the type of forces that it can carry. To identify the relevant structural properties that give rise to isostatic states it is useful to analyse a simple example first. Consider a framework of many thin struts connected rigidly at arbitrarily positioned junctions. It is well known from Maxwell's work [14] that for such structures to be statically determinate the mean number of elements connecting at the junctions, $\bar{z}$ (also known as the coordination number, the degree or the valency), has a particular value, z_c [15] when averaged over all junctions [16]. When this conditions is met the number of force components in the struts is equal to the number of balance equations that one can construct for the junctions. In three space dimensions $z_c = 4$ if the junctions can support torque moments and $z_c = 6$ if they cannot. The corresponding values for two-dimensional structures are $z_c = 3$ and $z_c = 4$, respectively. Frameworks with $\bar{z} < z_c$ are mechanically unstable and would rearrange spontaneously under the influence of a load. In frameworks where $\bar{z} > z_c$ not all the forces can be determined from the balance equations and additional conditions are required. Consequently, the global stress transmission in such structures is not perfectly isostatic, but may resemble in some regions the behaviour of conventional solids [17].

The understanding of stress transmission in isostatic media has improved considerably in recent years, particularly in the context of granular assemblies and cellular solids [18][19]. Of particular significance to the present discussion is a recent development of isostaticity theory for cellular solids [20][19]. The theory starts from the discrete Newton's equations for isostatic structures and provides a *continuous* description of the equations that govern the coarse-grained stress field. A detailed description of the theory can be found in references [19]-[21]. In the following only its salient points for two-dimensional systems are reviewed.

The closure equation of isostaticity theory in two dimensions comes from a *local* torque balance condition. This condition relates between the local stress and a specific *fabric tensor* p^v_{ij}, which characterises the local details of the structure (see figure 3). For example, for a planar framework of struts (denoted by the dotted lines in figure 3), these quantities are computed from

$$p^v_{ij} = \frac{1}{2}\sum_{c=1}^{3}\left(r_i^{cv}R_j^{cv} + R_i^{cv}r_j^{cv}\right) \ , \tag{4}$$

where i, j are indices that run over x, y and the vectors $\vec{r}^{cv}$ connect at the mid-points of the struts that emenate from junction (vertex) v. These vectors circulate the junction in the anti-clockwise direction and make loops around the voids, which they circulate in the clockwise direction (see figure 3). The vectors $\vec{R}^{cv}$ point from the centre of the triangle of vectors around v to the centre of one of its neighbour loops c. This fabric tensor is symmetric, $p^v_{ij} = p^v_{ji}$, and discrete, namely, it takes a specific value at each junction. The coarse-graining of this tensor leads to a continuous description of the structure, $p_{ij}(\vec{r})$. In terms of this tensor the closure equation in two dimensions is

$$p_{yy}\sigma_{xx} + p_{xx}\sigma_{yy} = 2p_{xy}\sigma_{xy} \ . \tag{5}$$

Eqs. (1)-(3) and (5) form the stress field equations for two-dimensional continuous isostatic media. Although the field equations seem difficult to analyse in their present form it is possible to decouple them, using an expansion in the gradients of the fields p_{ij}, which turn out to decrease strongly with lengthscale. To lowest order, this gives the following explicit equations for the stress components [19]

$$\left(p_{xx}\frac{\partial^2}{\partial x^2} + 2p_{xy}\frac{\partial^2}{\partial x \partial y} + p_{yy}\frac{\partial^2}{\partial y^2} \right) \sigma_{ij} = f_{ij} \ , \tag{6}$$

where the terms f_{ij} are known functions of the components of the fabric tensor and of the gradients of the external forces $\vec{g}$. The exact derivation of eq. (6) is not essential for the present discussion; what is significant is that it is hyperbolic! This means that the solutions of eq. (6) for localised stimuli do not spread uniformly, but rather propagate along *narrow channels.* The hyperbolic field equations and their solutions are termed isostaticity theory.

Let us illustrate the narrow-channel solutions in a particular system. Consider an isostatic medium occupying the semi-infinite plane $x \geq 0$, as sketched in figure 4. Let us presume that on the boundary of this system, $x = 0$, there acts a localised stimulus consisting of a force in the x-direction. Without loss of generality, choose the point around which the force is localised as $A = (0, 0)$. The boundary data is most conveniently represented in terms of stresses, $\sigma_{xx}(x = 0, y) = U(y)$, and their gradients, $\partial_x \sigma_{xx}(x = 0, y) = V(y)$, where $U(y)$ and $V(y)$ are arbitrary functions that, to mimic localised stimuli, are narrowly distributed around A. The imposition of the boundary data on σ_{xx} and $\partial_x \sigma_{xx}$ is chosen for its consistency with the hyperbolic nature of eq. (6). To make contact with experimentally measured forces, note that if the stimulus acts over a boundary section of size d then the force F_x in the figure is $\int_{-d/2}^{d/2} \sigma_{xx} dy$. For simplicity, we also assume that the external field $\vec{g}$ is constant in space (e.g. gravity). The general solution of the field equations under this stimulus is

$$\sigma_{xx} = \frac{1}{2}\left[U\left(\frac{S^0}{p^0_{xx}}\eta\right) + U\left(\frac{S^0}{p^0_{xx}}\zeta\right) \right] + \frac{1}{2}\int_\eta^\zeta \left[V(\frac{S^0}{p^0_{xx}}t) + \frac{p^0_{xy}}{p^0_{xx}} U'(\frac{S^0}{p^0_{xx}}t) \right] dt \ , \tag{7}$$

where $S = \sqrt{p_{xy}^2 - p_{xx}p_{yy}}$ and $U' = dU(s)/ds$ is the derivative of U with respect to its argument. S^0 and p^0_{ij} stand for the value of these quantities along the boundary $x = 0$. The coordinates η and ζ are linear combinations of x and y

$$\begin{aligned} \eta &= -\left(1 + \frac{p_{xy}}{S}\right) x + \frac{p_{xx}}{S} y \\ \zeta &= -\left(1 - \frac{p_{xy}}{S}\right) x + \frac{p_{xx}}{S} y \ , \end{aligned} \tag{8}$$

where the coefficients of x and y depend *only* on the geometric characteristics p_{ij}. The solution (7) shows that the boundary stimulus propagates into the medium via two characteristic lines, $y_\eta(x)$ and $y_\zeta(x)$, along which the values of η and ζ are constant. For concreteness, suppose a stimulus of magnitude σ^0 has a bell-like form and is loclised within an area of size d on the boundary,

$$U(y) = \sigma^0 e^{-y^2/2d^2} \quad \text{and} \quad V(y) = 0 \ , \tag{9}$$

The explicit solution for the stress field in the medium for this loading is

$$\sigma_{xx} = \frac{\sigma^0}{2}\left[\left(1 + \frac{p_{xy}}{S}\right) e^{-\frac{S^2\zeta^2}{2d^2 p_{xx}^2}} + \left(1 - \frac{p_{xy}}{S}\right) e^{-\frac{S^2\eta^2}{2d^2 p_{xx}^2}}\right] \ , \tag{10}$$

The solution consists of two bell-shaped peaks that propagate into the medium, as shown schematically in figure 4. The peaks of the signals are centered on the two lines

$$y_\eta = \frac{S + p_{xy}}{p_{xx}} x \quad \text{and} \quad y_\zeta = \frac{S - p_{xy}}{p_{xx}} x \ . \tag{11}$$

The forces that propagate along these lines can be calculated by first using eqs. (11) to find the local unit tangents to these lines, $\vec{t}_\eta$ and $\vec{t}_\zeta$, and then carrying out the following integrals $F_\eta = \int_{-d/2}^{d/2} \hat{\sigma} \cdot \vec{t}_\eta dy$ and $F_\zeta = \int_{-d/2}^{d/2} \hat{\sigma} \cdot \vec{t}_\zeta dy$.

This class of nonuniform solutions provides an effective mechanism for transmission of the force from the stimulus point into the cell via two narrow channels, along which the forces *hardly attenuate*. Although the trajectory of a channel may fluctuate locally, its general direction is well defined. For example, the trajectories drawn in figure 4 were computed for *random* values of the p_{ij}'s, chosen from a broad distribution. The amplitude of the fluctuations was 0.1 with a standard deviation of 0.08. Yet, the lines appear almost perfectly straight. This feature makes it possible to identify the origin of the stimulus force anywhere along the transmission channel from the local gradient. Another significant feature of this model is that, given knowledge of the boundary stimulus at point A and of the (possibly temporal) structure of the network, it is possible to determine *apriori* the exact paths that the forces would take from A. This has possible ramifications on the dynamics of reorganisation of the CSK (see discussion in the concluding section). It is also possible to *terminate* one of the paths by local reorganisation of the CSK structure, as will also be discussed below, giving the cell even greater control over the force transmission.

A caveat to the analysis is that, at present, isostaticity theory has only been developed explicitly in two dimensions. The three-dimensional theory, where three 'missing' conditions are required to close the stress equations, has not yet made an appearance in the literature. Nevertheless, these equations have been derived recently by this author, as will be reported shortly. Initial calculations of simple networks indicate that the three-dimensional equations support a similar type of nonuniform solutions.

The above discussion provides a potential dream model for intracellular stress transmission: (i) it is conveniently continuous, yet it describes communication of forces via narrow channels; (ii) the model gives rise to a nonuniform transmission of signals that describes the action at a distance observed experimentally; (iii) forces hardly attentuate along the channels, giving very good signal-to-noise ratios; (iv) the narrow channeling makes it possible for the cell to exercise good control over the signal; (v) the predictability of force path directions both enables the modeller to predict the exact stresses that develop in the CSK and it allows the cell itself to exploit this feature as an input for the reorganisation dynamics of the CSK structure (see below);

It follows that an isostatic, or near-isostatic, structure of the CSK would make possible a good control over mediation of forces and provide explanation to several difficult experimental observations. But is the CSK close to an isostatic state? This author could find no direct experimental evidence that would provide a clear cut answer to this question. Such evidence is essential if we wish to benefit from the advantages that this model offers and use its predictive power. To provide a basis for testing this issue it is important to quantify the structural characteristics that need to be observed if the CSK is in such a state. This is done in the next section.

The conditions for isostaticity of the CSK

As mentioned, the CSK consists of three primary types of filaments: AFs, IFs, and microtubules. It is commonly believed that the former two support only tensile forces, but it is worth commenting that this depends on both the magnitude of the applied forces and the structure. Bending forces can be ignored only if they give rise to sufficiently weak torque moments. This means that, to cover all angles, the modelling should take into consideration cases when bending forces drop to a level that some sections of the AFs or IFs can support. When, or if, that happens those particular sections must also be regarded as stiff elements. The complication arises from the dependence of the type of behaviour on both force magnitude and the length of elements. For completeness, all possible scenarios will be discussed below.

In contrast, microtubules, which are hollow and stiff cylindrical filaments, are able to support all types of forces including relatively large compressive forces and bending forces. Consequently, they are usually regarded as struts. Note that struts need not be straight in the framework.

AFs typically form junctions of two kinds: (i) cross-links between two linear filaments, in which case four arms come out of the junction and it is termed quadrivalent; (ii) branching points, in which case three arms emanate from the junction and it is termed trivalent. The CSK framework can be regarded as a collection of elements, each extending between two neighbouring junctions. Any one filament may consist of a number of elements, e.g. the filament in figure 5 which includes elements α, β and γ.

An element transmits a force between junctions - cable elements transmit only longitudonal (tensile) forces while struts transmit forces that have both longitudonal (tensile or compressive) and transversal (bending) components. The bending components give rise to torque moments around the junctions. A force vector between two junctions is therefeore characteristic of the element between them and is termed in the following element force. For the purpose of the following analysis, elements that transmit no force at all between junctions are not considered to be part of the framework.

For the framework of filaments to be isostatic under external loading, even only temporarily, the forces that develop in the elements must match the number of equations that can be constructed around the junctions. We consider three possible scenarios in increasing levels of generality.

A. Suppose that all the junctions between the filamentous components can support torque moments. This would be the case if most of the fillaments could support bending forces. In this case many junctions would connect to relatively stiff struts, as sketched in figure 5b. This scenario is presented here mainly for completeness and to illustrate the rationale of the argument for a relatively straightforward case. Nevertheless, as mentioned above, it may also have practical relevance if some of the forces that develop in the CSK under working conditions are sufficietly weak. While such a possibility is unlikely to occur in bead pulling experiments, where forces are of order of hundreds of pN, such a situation cannot be ruled out apriori in all cells under working conditions.

Consider then a framework of N junctions in mechanical equilibrium under an arbitrary set of external forces on its boundary. The external loading gives rise to forces in the elements, as discussed above. If the structure is in an isostatic state then the forces can be determined from balance equations. It is convenient to define the following (dual) structure. Around every junction draw straight lines connecting the midpoints of the elements that connect to it, as sketched in figure 6. These lines make edges of polyhedra that surround the junctions and every polyhedron is rigidly connected at its corners with its neighbours. Under the same external loading, the frameowrk of tetrahedra transmits exactly the same forces as the CSK with the force between neighbouring polyhedra v and v' being identical to the element force, $f_{vv'}$, between junctions v and v' (see figure 6). Let us count the number of unknown force components. The element forces are vectors, which means that at every contact between neighbouring polyhedra there are three unknowns to determine. The number of unknowns is then three times the number of struts, $N_{unknown} = 3S$. Denoting the valency of junction v by z_v and considering $N \gg 1$ [16], the total number of strut elements between junctions is

$$N_{elements} = \frac{1}{2} \sum_{v=1}^{N} z_v \equiv \frac{1}{2} N \bar{z} \ , \tag{12}$$

where $\bar{z}$ is the mean valency per junction. Therefore, there are altogether $3N\bar{z}/2$ unknowns.

To count the number of equations that can be constructed for these unknowns, note that each polyhedron is in mechanical equilibrium under the forces applied on it by its neighbours. Then, it must satisfy both balance of forces (three equations for the three space directions) and balance of torque moments (three equations for the three axes of rotations in three dimensions). This gives six equations per polyhedron and altogether $6N$ conditions. To be statically determinate we must have $3N\bar{z}/2 = 6N$, which gives that the only requirement that the structure should satisfy for it to be isostatic is $\bar{z} = 4$. It should be noted that the requirement is on the *mean valency* rather than on the valency of every junction and therefore it is quite unrestrictive.

B. In the second scenario we relax the above assumptions and suppose that the range of forces is such that most, or all, of the AFs and IFs *cannot* support bending forces. In this case these elements can be regarded as cables, supporting only tensile forces. An element between any two neighbour junctions can be either a cable or a strut. Presume for the moment that the fraction of struts is sufficiently high so that every junction connects *at least* two struts. This means that all the junctions can support torque moments (see, e.g. figure 5c). The likelihood of this scenario is again subject to occurrence of sufficiently weak intracellular forces in some elements under working conditions, as discussed above.

The difference between this and scenario A is that now not all element forces have three unknowns. Rather, because the tensile forces in the cables align in the direction of the element and the structure is given then only the magnitudes of the cable forces are unknown. This leaves *one unknown per cable.* Struts support forces that can point in arbitrary directions, giving three unknowns per strut as before. Denoting the total number of strut and cable elements in the structure S and C, respectively, the total number of unknowns is then

$$N_{unknowns} = 3S + C \ . \tag{13}$$

The total number of elements between junctions is again $\bar{z}N/2$,

$$N_{elements} = C + S = \frac{\bar{z}N}{2} \ . \tag{14}$$

Since every junction can support a torque moment then, to be in mechanical equilibrium, each polyhedron must satisfy six equations as before, giving a total of $6N$ equations. It follows that for the structure to be isostatic the following equality must be satisfied

$$6N = 3S + C \ . \tag{15}$$

Solving from relations (14) and (15) for S and C, we obtain the following conditions for isostaticity

$$S = \left(3 - \frac{\bar{z}}{4}\right) N \quad \text{and} \quad C = 3\left(\frac{\bar{z}}{4} - 1\right) N \ . \tag{16}$$

We can check this relation for consistency by noting that for networks made only of torque-carrying elements (struts) $C = 0$ and we recover the condition of the previous case, $\bar{z} = 4$.

Recall that this scenario requires that the number of struts around every vertex is at least two. Since every strut is party to two junctions then this places bounds on the total number of struts, $2N \geq S \geq N$. Comparing this inequality with eq. (16) gives $4 \leq \bar{z} \leq 8$. The lower bound is in fact the condition for mechanical stability at all; for values of $\bar{z} < 4$ the structure cannot be in equilibrium and must rearrange under the application of any small force. As we saw, this value is consistent with all elements being struts. At the opposite extreme, if all elements were cables then, as we shall see below, the mean valency for static determinacy is $\bar{z} = 6$. Thus, $\bar{z} < 8$ does not yield new information.

C. The third scenario is also the most general and probably the most realistic. The networks are now allowed to contain some junctions that cannot support any torue moment. Let these junctions comprise a fraction x of the total number of junctions N. The key difference between this and the previous scenario is that in mechanical equilibrium these xN junctions can only provide *three equations* of force balance each. The remaining $N(1-x)$ junctions can still provide six balance equations each as before - three of force and three of torque. Therefore, the total number of equations available to determine the forces is now $6N(1-x)+3Nx = 3N(2-x)$. As before, there are three unknown force components to determine at each strut, one unknown to determine at each cable and relation (14) still holds. However, we must be careful with the counting of C. Some of the xN junctions, which do not support torques, may still be connected to the end of exactly one strut. Being the only strut at a junction means that the strut can only support a longitudonal force, tension or compression. It follows that, in spite of being a stiff element, this strut only gives rise to one unknown force component. Therefore, for the purpose of the analysis of this scenario, such a strut should be counted within C. Requiring now that the number of equations be equal to the number of unknowns gives

$$3N(2-x) = 3S + C \ . \tag{17}$$

Solving relations (14) and (17), gives that for static determinacy there should be the following numbers of struts and cables

$$S = \frac{6(2-x)-\bar{z}}{4}N \quad \text{and} \quad C = \frac{3\bar{z}-6(2-x)}{4}N \ . \tag{18}$$

We can check this result for consistency. When $x = 1$ there are no torque carrying junctions at all, which means that $S = 0$. Therefore, all elements carry forces that align along the direction of the filament, and eqs. (18) give $\bar{z} = 6$. This is the tensegrity limit because the structure is made mostly of cables with occasional struts that are under compression between junctions. The opposite limit $x = 0$ reproduces eqs. (16).

If we denote the relative fraction of cables and struts in the structure α and $1-\alpha$, respectively, then we can deduce from relations (18) that the mean valency is

$$\bar{z} = \frac{6(2-x)}{3-2\alpha} \ . \tag{19}$$

Relation (19) describes a surface in the three-dimensional space spanned by x, α and $\bar{z}$, as shown in figure 7. However, the values of α and x must be consistent with one another and therefore are not entirely independent. For example, when $x = 0$ there is an upper bound on α, which must lie well below 1, and when $x = 1$ there is a lower bound on α, which must be well above 0. This imposes constraints on the surface shown in figure 7 in the sense that not all regions on it are physically realizable. Nevertheless, the relation shows that the mean valency must lie between the values $\bar{z} = 4$, when $x = 0$ and $\alpha = 0$, and $\bar{z} = 6$, when $x = 1$ and $\alpha = 1$, a prediction that can be tested experimentally.

Discussion and conclusion

To conclude, this paper has examined the paradigm that underlies modelling of intracellular stress transmission. It has been shown that conventional continuous models do not fail merely because of being continuous, but because of the Saint-Venant compatibility condition built into the equations that govern the stress field. This condition necessitates the introduction of deformation-based information and is directly responsible for the uniformisation of stress fields away from a localized stimulus. It is this basic feature that makes these models inadequate for describing action at a distance.

It has also been argued that turning to discrete models as a way to overcome this difficulty is based on two misguided ideas: that discrete models would automatically give rise to nonuniform fields and that all continuous models would automatically give rise to uniformisation of stresses. Both these misconceptions have been put straight - (i) discrete models will give rise to stress fields that dissipate on scales larger than the mesh size unless their structures satisfy specific conditions; (ii) continuous models may give rise to nonuniform force channeling as long as the field equations are hyperbolic. Thus, while discreteness may be a necessary condition for the focusing and directing of forces on the element scale, it is by no means sufficient to maintain directionality over large distances.

A new model has been proposed, based on the idea that action at a distance and channeling of force stimuli is possible if considerable parts of the CSK structure were isostatic. Isostaticity theory has been briefly reviewed in two dimensions and it has been shown to be indeed a promising model for the transmission of intracellular forces due to several advantages: (i) it offers the convenience of continuous modelling and the ability to describe stresses on lengthscales larger than the size of the mesh of the CSK; (ii) it gives rise to nonuniform stresses and to propagation of forces into the cell via narrow channels, which explains the good control that cells have over transmission of mechanical signals; (iii) it explains the experimental observations of action at a distance; (iv) given a specific force stimulus, the model makes it possible to predict the path along which the force focuses as it propagates into the cell.

For a large region of the CSK to be isostatic it must satisfy several conditions, the most important of which is that its mean valency $\bar{z}$ must have a specific value. This value depends on the ratio of the molecular elements in the strusture that are able to support bending forces (struts) to those that can support only tensile forces (cables). Correspondingly, the mean valency dependends on the fraction of junctions that are able to support torque moments. The structural conditions have been explicitly given for several possible scenarios of cable to strut distributions. It has been shown that, to be perfectly isostatic in any scenario, the mean valency must lie between $\bar{z} = 4$ (when all the elements are struts) and $\bar{z} = 6$ (when no junction can support torque moments).

This analysis highlights an issue that has no analogue in convonetional models. Whether a structure is isostatic depends crucially on the distribution of cable and strut elements that it is composed of. But AFs and IFs, which definitely behave as cables under typical forces of bead pulling experiments, may behave as struts under much weaker forces. This raises an important question: could intracellular forces under working conditions be sufficiently weak to allow fractions of AFs and IFs to support non-negligible bending forces, at least in some cells? If so then scenario A and B could be more relevant than currently believed for the representation of real CSK networks. Moreover, if this turns out to be the case, even only partially, then bead pulling and twisting experiments may be missing the point by measuring at force magnitudes that wash out very relevant phenomena. Note that in conventional models the magnitude of the forces do not play any conceptual role, while for isostaticity theory they are essential. Thus, if forces in AFs and IFs under working conditions are sufficiently weak then conclusions based on experiments that involve applying much higher forces to cells should be re-examined.

It should be emphasised that for the cell to exert the type of control on stress transmission described here the CSK structure need not be precisely isostatic. It is not inconceivable that the structure of the CSK, which is constantly changing and reorganising, may only satisfy these conditions approximately and so be only nearly isostatic. The proximity to perfect isostaticity is measured by the discrepancy between the number of unknown forces and the number of balance equations that can be constructed for them. If the discrepancy is not too large compared to the number of junctions then the CSK consists of considerable regions that are locally isostatic. In these regions force transmission would be channeled along narrow paths. The smaller the discrepancy, the larger the isostatic regions, the closer the structure is to being isostatic and the longer the force channels. This would lead to minimal attenuation and cells would have better control over the mediation of forces. Ultimately, the proximity of the CSK to an isostatic state is determined by the combination of the mean valency and the ratio of cables to struts. If the mean valency is too low relative to $\bar{z}$ then a stable structure cannot be sustained at all. If, on the other hand, too many junctions connecting microtubular elements have high valencies then the framework is over-connected and too far from an isostatic state. This would lead to deterioration of the control over force transmission, effecting a behaviour characteristic of conventional materials. Forces applied at the focal adhesion points would then diffuse and attenuate as they penetrate the cell. Regular periodic structures are usually in this regime due to their high symmetry. The possibility to control closeness to isostatic states is a considerable advantage from a designer's point of view - the dependence on a global mean of the valency means that the ability to channel and control forces is not over-sensitive to local structural fluctuations.

This leads to an interesting speculation: an effective strategy for the cell may be to maintain a CSK structure that is not exactly isostatic but only predominantly so. This allows to have judiciously selected small regions of locally high mean valencies that behave either elastically or visco-elastically. By controlling the size and nature of such regions it is possible both to maintain good control over the paths along which forces propagate and to admit pereferntial deformations that can accommodate other cell functionalities not addressed here. Entertaining small 'elastic' regions has another advantage. Recall that in response to a localised force stimulus an isostatic medium generates *a pair* of force channels. But the cell only needs one such a signal to reach the vicinity of the nucleus. Placing a high-z region in the path of one of the channels effectively terminates the focused force and dissipates it. Thus, the manipulation of local connectivity provide several important mechanisms to control force propagation into the cell.

Many of these ideas can be tested experimentally. One test would be a determination of the proximity of CSK structures to isostatic states and the effects that such proximity has on the stress transmission through focused channels. A relatively straightforward way to obtain quantitative data on this issue would be by measurements of bending forces that AF-AF and AF-IF junctions can support.

These measurements should aim at providing an estimate of the dependence of the bending forces on both the length of the filamentous elements and on the magnitude of the applied forces. These data may already exist in the literature. Another way to address this issue would be by measurements of the mean valency $\bar{z}$ and the relative fractions of cables to struts α. Once this information is known it is possible to invert relation (19) to obtain an estimate for x:

$$x = 2 - \frac{(3 - 2\alpha)\bar{z}}{6} \ . \tag{20}$$

This value can be checked for consistentcy with α according to the constraints discussed in the text and the consistency, or its absence, can be used to test the theory.

Another set of observations could be carried out to estimate the fraction of junctions $(1 - x)$ where more than two microtubular elements meet. It should also be possible to determine directly the total fraction of struts to cable elements in the structure (keeping in mind that in some cases microtubular elements under compression carry no torques but only longitudonal forces). This information may also exist already in the literature in some form or another, but even if it does it should probably be re-assessed taking into consideration the definition of elements in this analysis as filaments between neighbouring junctions. Another set of experiments should focus on the dynamics of reorganisation of the structure with an attempt to determine whether one of the aims of the process is to accomplish local isostaticity (or perhaps undermine it, as discussed above) and how does the reorganization correlate with force channeling.

If the picture proposed here is correct and isostatic states indeed play a significant role in intracellular stress transmission then it must have implications regarding the dynamics of the CSK. In particular, one aim of reorganization would then be to control force transmission through control of local isostaticity. A potential mechanism may be the following. As a force is applied to a cell surface (integrin) receptor it initially propagates into the cell roughly in the direction of the nucleus. Then actin filaments are polymerised judiciously, following mechano-chemical cues, rearranging the structure into a locally isostatic state which channels the force more precisely towards its intended destination.

From a designer's point of view, such structural adjustments best be relatively localised for several reasons: one is that the reorganization process should be inexpensive in terms of energy and raw materials required, another is that the larger the reorganization the larger the chemical activity involved and therefore the longer the response time, and finally that a large reorganization for this purpose may adversely affect other ongoing functions of the CSK, such as being a highway for enzyme motors. Thus, maintaining 'dynamic local isostaticity' could be more efficient than maintaining a global isostatic structure.

Another point to note is that a constant change of the entire CSK structure is not very economical. To use this mechanism effectively, large parts of the CSK network must remain relatively fixed, or change on relatively long timescales. It is around these stable parts that the CSK structure would be dynamically manipulated. It is tempting to conjecture that this may be one of the roles of the intermediate filaments

- to act as relatively stable elements around which rearrangement is much more dynamic - but this needs to be checked. This issue, as well as all the speculations on the dynamics of rearrangement, are amenable to experimental testing.

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Figure Captions:

- 1. A localised force stimulus is applied at point A and is felt at point B through the cytoskeleton that extends between these points. Two modes of propagation of the force are sketched: (a) directed propagation, where the force is mediated through a narrow channel; (b) diffusive propagation, where the stress becomes more uniform as the distance from A increases. In case (b) the force felt at point B is a small fraction of the original stimulus because the stimulus force is supported practically by a large surface which corresponds in the sketch to the thick arc.

- 2. A ladder of weight W standing on a rough floor is leaning against a smooth wall. The friction coefficient of the floor is $\mu > 0$, whilst that of the wall is $\mu = 0$. The floor and the wall react to the forces that the ladder applies on them via the forces $\vec{f}_1$, $\vec{f}_2$ and $\vec{f}_3$. In mechanical equilibrium the three forces can be determined by balance conditions alone - of forces and of torque moments. The forces are independent of the compliance of the material that makes the ladder.

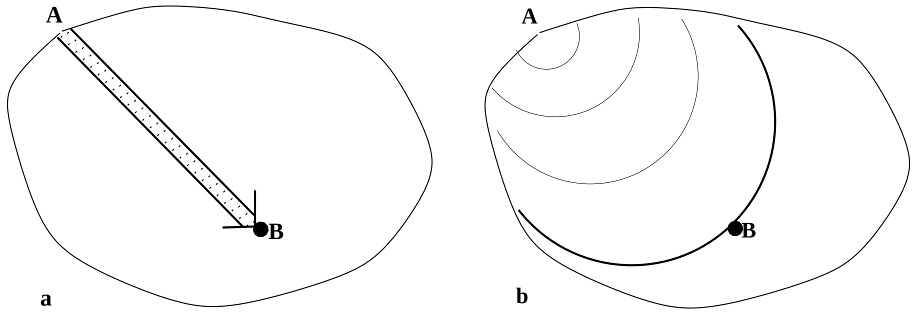
- 3. An example of the structural characterisation in a two-dimensional framework of struts. The vectors $\vec{r}^{cv}$ connect midpoints of the struts around junction v, circulating in the anti-clockwise direction. The vector $\vec{R}^{cv}$ extends from the centre of triangle v to the centre of one of its neighbour loops, c. The symmetric part of each term in the tensor $\hat{C}^v = \sum_l \vec{R}^{cv}\vec{r}^{cv}$ gives the components of the fabric tensor p_{ij} which couple to the stress tensor in eq. (5). This equation and the balance conditions (1)-(3) form a closed set of stress field equations.

- 4. An example of the propagation of a bell stimulus at the boundary into a two-dimensional isostatic medium. The medium is continuous and the magnitude of σ_{xx} is plotted at discrete points in the material. The values of the components of the geometric tensor are taken to be random. Note that: (i) the propagation need not be symmetric around $y = 0$ and (ii) that the amplitudes of the forces along the two channels need not be the same.

- 5. (a) One filament may consist of several elements. Elements are sections of the filament that are separated by junctions and transmit forces between them, e.g. α, β and γ. (b) A junction connecting four rigid filaments can transmit bending forces and therefore torque moments. (c) A junction may join both cables and struts.

- 6. The construction of the framework of polyhedra. The edges of a polyhedron consist of lines extended between midpoints of elements meeting at the junction. Every polyhedron is considered to connect rigidly to its neighbouring polyhedra. There are three conditions of force balance around every polyhedron and, depending of the nature of the elements entering the junction, one may also construct three conditions of torque balance.

- 7. For the CSK structure to be isostatic the mean valency $\bar{z}$ is a given function of the fraction of torque carrying junctions, x, and the fraction of cable elements α in the framework. This function describes a surface in the three-dimensional space spanned by x, α and $\bar{z}$.



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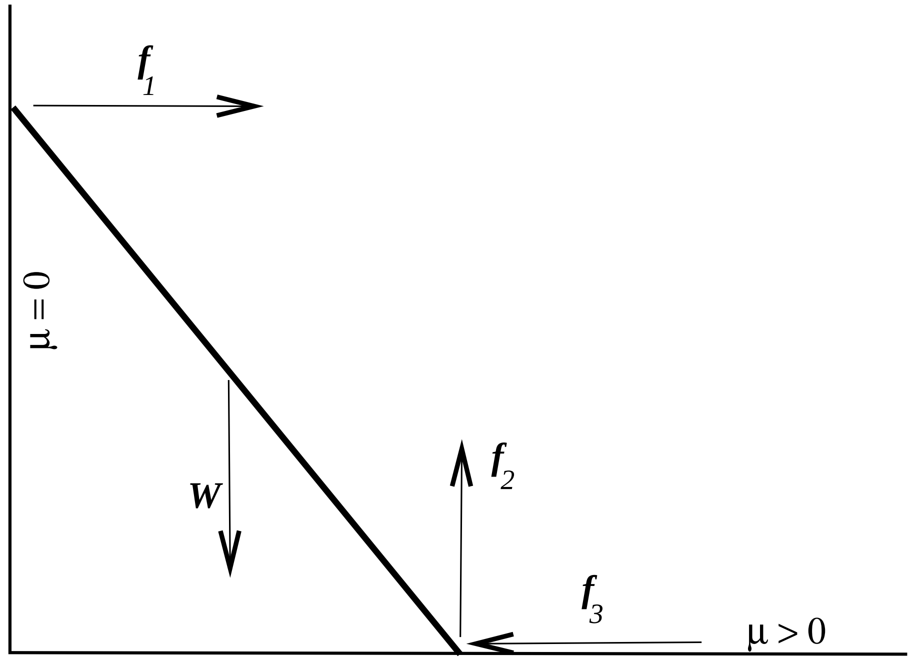

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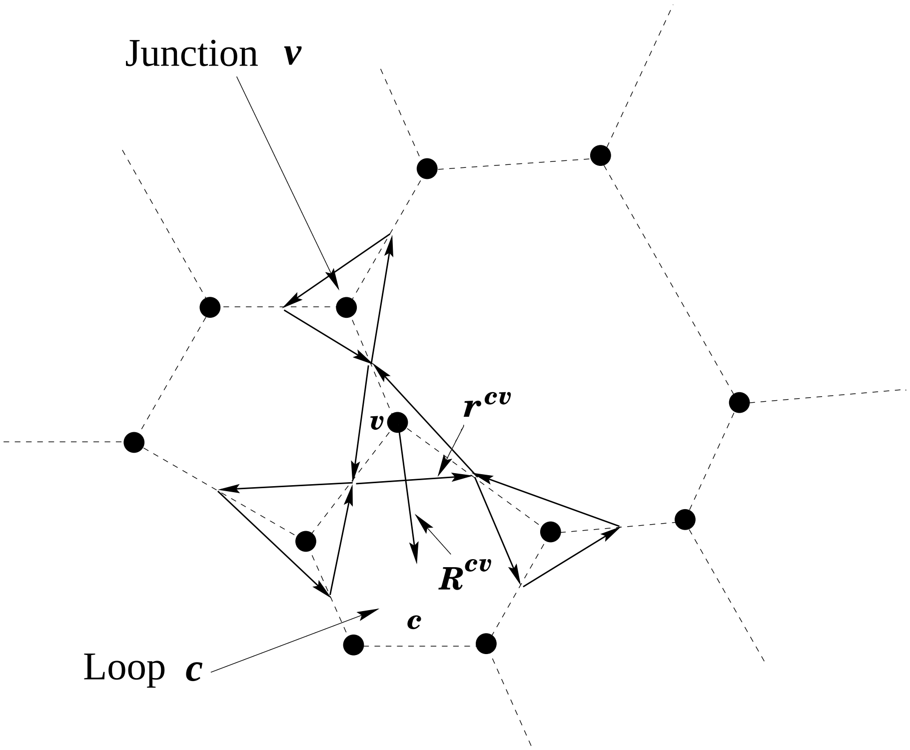

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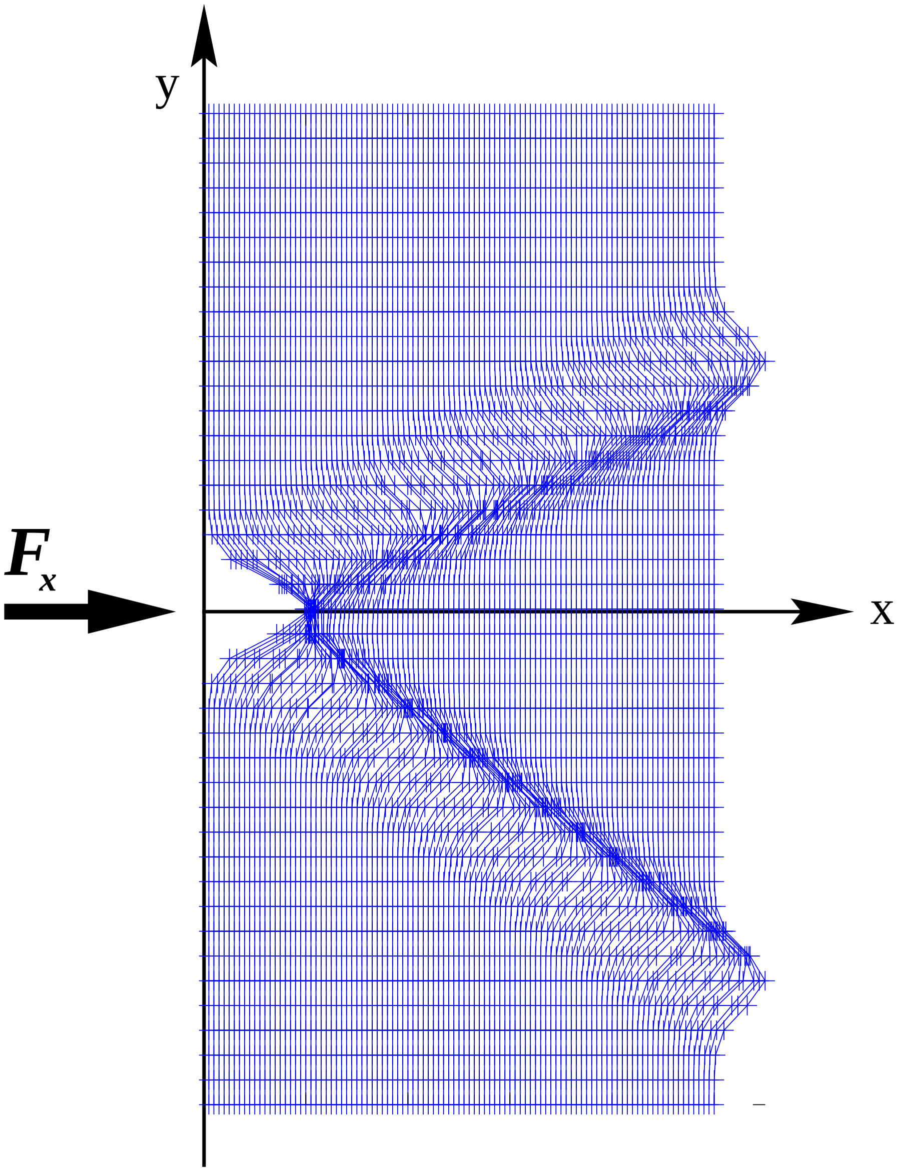

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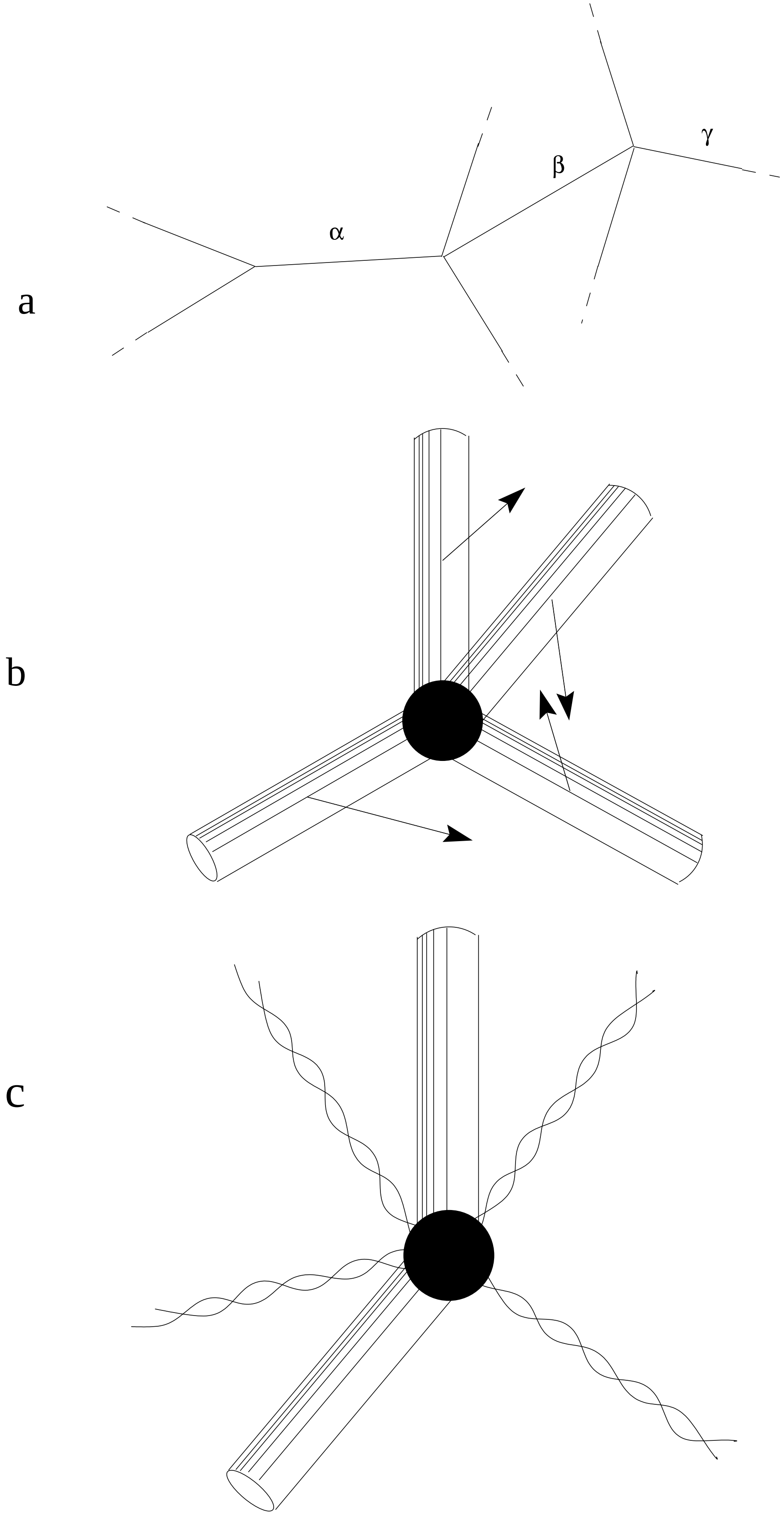

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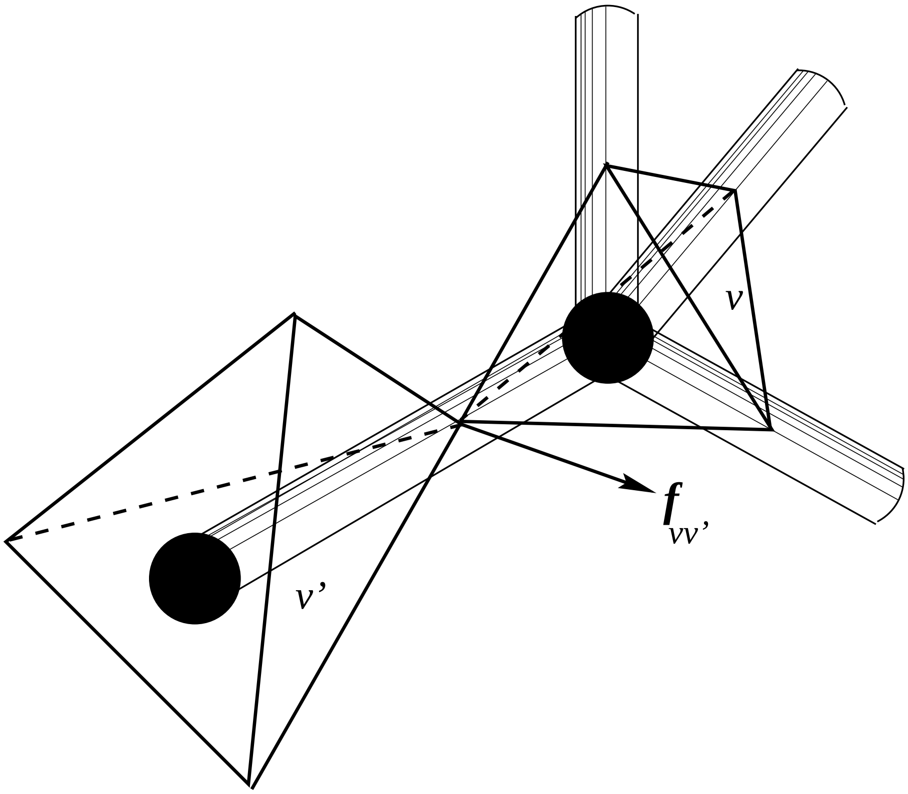

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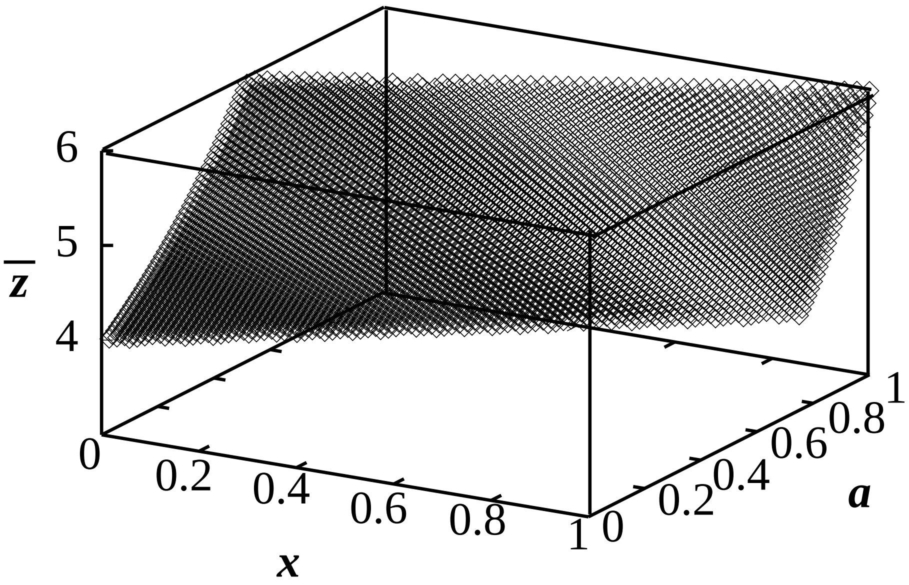

Figure 7: